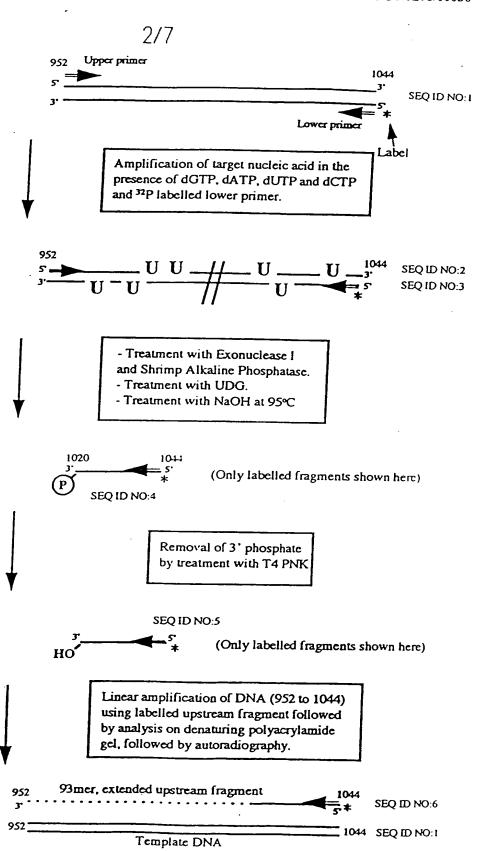
Phosphodiester bond at 3' side of AP site

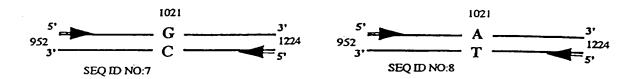
FIG 1

DOEZEZEG TOBOO



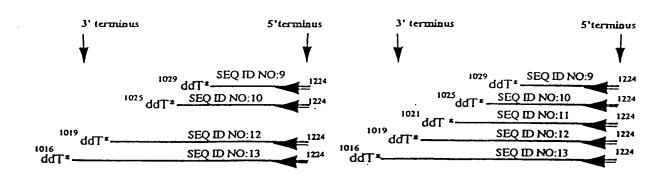
3/7

- Amplification of normal and mutanttarget nucleic acid in the presence of dGTP, dATP, dCTP and 1/20 ratio of dUTP to dTTP.



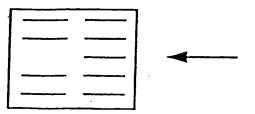
- Treatment with Exonuclease I and Shrimp Alkaline Phosphatase.
- Treatment with UDG.
- Treatment with NaOH at 95℃
- DNA is precipitated.
- Treatment with T4 PNK

Extension of the upstream fragments generated above in the presence of ³³P-labelled ddTTP* and unlabelled ddGTP, ddATP and ddCTP.

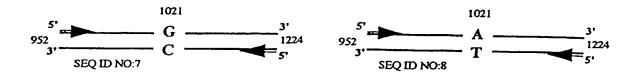


Only some fragments corresponding to cleavage at U incorporation sites surrounding the mutation site are shown here.

Detection of extended labelled fragments by PAGE and autoradiography

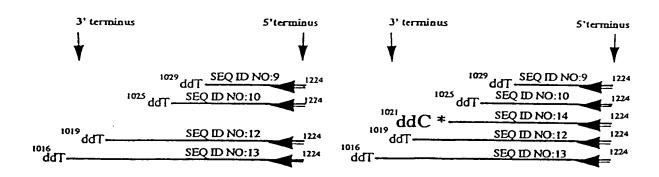


- Amplification of normal and mutanttarget nucleic acid in the presence of dGTP, dATP, dCTP and 1/20 ratio of dUTP to dTTP.



- Treatment with Exonuclease I and Shrimp Alkaline Phosphatase.
- Treatment with UDG.
- Treatment with NaOH at 95°C
- DNA is precipitated.
- Treatment with T4 PNK

Extension of the upstream fragments generated above in the presence of ³³P-labelled ddCTP* and unlabelled ddGTP, ddATP and ddTTP.



Only some fragments corresponding to cleavage at U incorporation sites surrounding the mutation site are shown here.

Detection of extended labelled fragments by PAGE and autoradiography

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6390 Upper primer

Mutation site

SEQ ID NO:15

6443

- 5. AACTTGTGGTAGTTGGAGCTGGTGGCGTAGGCAAGAGTGCCTTGACGATACAGC 3.
- 3. TTGAACACCATCAACCTCGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5.

T

Lower primer

Amplification of target nucleic acid in the presence of dGTP, dATP, dUTP and dCTP.

SEQ ID NO:16

Amplified normal allele

- 5' AACTTGTGGTAGTTGGAGCTGGUGGCGUAGGCAAGAGUGCCUUGACGAUACAGC 3'
- 3' UUGAACACCAUCAACCUCGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5'
 SEQ ID NO:17

SEQ ID NO:18

Amplified mutant allele

- 5' AACTTGTGGTAGTTGGAGCTGAUGGCGUAGGCAAGAGUGCCUUGACGAUACAGC 3'
- 3' UUGAACACCAUCAACCUCGACUACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' SEQ ID NO:19

- Treatment with Exonuclease I and Shrimp Alkaline Phosphatase.
- Treatment with UDG.
- Treatment with Endo IV.

SEQ ID NO:20

Normal upstream fragment

3'CGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5'

SEQ ID NO:21

Mutant upstream fragment

3'ACCGCATCCGTTCTCACGGAACTGCTATGTCG 5'

6

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SEQ ID NO:23 A Reverse primer 5 GCTGTAAACGACGCCAGTTTCAT 3 SEQ ID NO:22 Synthetic template No. 1 5 CCTGTAAACGACGGCCAGTTTCATGCAGGGCTGGAGTCGTAGGCAAGAGTGCCTTGACGATACAGC 3 TCTCACGGAACTGCTATGTCG 5. CGACCACCGCATCCGT Normal upstream fragment **SEQ ID NO:20** PCR amplification in presence of α³²PdCTP followed by denaturing PAGE SEQ ID NO:24 3 CGACATTTGCTGCCGGTCAAAGTACGTCCCGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5 66cmer B Reverse primer SEQ ID NO:23 5'GCTGTAAACGACGGCCAGTTTCAT 3' Synthetic template No.2 SEQ ID NO:25 5 CCTGTAAACGACGGCCAGTTTCATGCAGGATCCATGGCGTAGGCAAGAGTGCCTTGACGATACAGC 3 XXXXX 3'CGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' Normal upstream fragment SEQ ID NO:20 PCR amplification in presence of \alpha^32PdCTP followed by denaturing PAGE SEQ ID NO:23 Reverse primer 5'GCTGTAAACGACGGCCAGTTTCAT 3' SEQ ID NO:25 Synthetic template No.2 5' GCTGTAAACGACGGCCAGTTTCATGCAGGATCCATGGCGTAGGCAAGAGTGCCTTGACGATACAGC.3' 3' ACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' Mutant upstream fragment SEQ ID NO:21 PCR amplification in presence of α32PdCTI followed by denaturing PAGE SEQ ID NO:26 3 CGACATTTGCTGCCGGTCAAAGTACGTCCTAGGTACCGCATCCGTTCTCACGGAACTGCTATGTCG 5 66mer D SEQ ID NO:23 Reverse primer 5'GCTGTAAACGACGGCCAGTTTCAT 3' Synthetic template No.1 SEQ ID NO:22 5' GCTOTAAACGACGGCCAGTTTCATGCAGGGCTGGAGTCGTAGGCAAGAGTGCCTTGACGATACAGC 3' X X
3'ACCGCATCCGTTCTCACGGAACTGCTATGTCG 5' Mutant upstream fragment **SEQ ID NO:21** PCR amplification in presence of a³²PdCTP followed by denaturing PAGE Mismatches denoted by X ³²P label denoted by *



PCT/IE98/00030

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```
SEQ ID NO:27
         A
                                 Template oligo 1
                              GGTAGTTGGAGCTGGTGGCG
                                                                   SEQ ID NO:20
                                          allille
                          CCATCAACCT'
                                          CGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG
                          3' Reporter 5'
                 oligo 1
SEQ ID NO:28
                                                             Normal upstream fragment 5'
                                          Ligation reaction followed by
                                          denauring PAGE
                                                              SEQ ID NO:29
                    3 CCATCAACCTCGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5
                                                  47mcr
       В
                                            SEQ ID NO:27
                                 Template oligo 1
                           GGTAGTTGGAGCTGGTGGCG
                                                                SEQ ID NO:20
                  AACCTCGACC*
                                       CGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG
                    Reporter 5'
                                                          Normal upstream fragment 5
                    oligo 2
           SEQ ID NO:30
                                        Ligation reaction followed by
                                        denaturing PAGE
                            AACCTCGACC • SEQ ID NO:30
3' 10mcr 5'
      C
                                        SEQ ID NO:31
                             Template oligo 2
                                             3.
                        TTGGAGCTGGTGGCGTAGGC
                                                         SEQ ID NO:21
                                   ACCGCATCCGTTCTCACGGAACTGCTATGTCG
                      Reporter 5'
                                               Mutant upstream fragment 5
                      oligo 2
           SEQ ID NO:30
                                      Ligation reaction followed by
                                      denaturing PAGE
                                              SEQ ID NO:32
              3' AACCTCGACCACCGCATCCGTTCTCACGGAACTGCTATGTCG 5'
                                        42mcr
     D
                                          SEQID NO:31
                             Template oligo 2
                        TTGGAGCTGGTGGCGTAGGC
                                                          SEQ ID NO:21
                                    mm
                     ATCAACCT
                                   ACCGCATCCGTTCTCACGGAACTGCTATGTCG
                     Reporter 5
                                                Mutant upstream fragment
                     oligo 1
           SEQ ID NO:28
                                     Ligation reaction followed by
                                     denaturing PAGE
                                                                     32P label denoted by *
                                                                      // denotes basepairing
                          CCATCAACCT • SEQ ID NO:28
FIG 7
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